

FILE 'REGISTRY' ENTERED AT 14:48:01 ON 19 SEP 2005

=> S RESTRICTION ENZYME/CN

L1 1 RESTRICTION ENZYME/CN

=> D

L1 ANSWER 1 OF 1 REGISTRY, COPYRIGHT 2005 ACS on STN

RN 9075-08-5 REGISTRY

ED Entered STN: 16 Nov 1984

CN Nuclease, restriction endodeoxyribo- (9CI) (CA INDEX NAME)

OTHER NAMES:

CN DNA restriction endonuclease

CN DNA restriction enzyme

CN E.C. 3.1.21.4

CN E.C. 3.1.4.32

CN Nuclease, deoxyribonucleic restriction endo-

CN Restriction endodeoxyribonuclease

CN Restriction endonuclease

CN Restriction enzyme

DR 37288-31-6

MF Unspecified

CI MAN

LC STN Files: ADISNEWS, AGRICOLA, BIOBUSINESS, BIOSIS, CA, CAPLUS, CEN,
CHEMLIST, CIN, CSCHEM, IFICDB, IFIPAT, IFIUDB, PROMT, TOXCENTER, USPAT2,
USPATFULL

Other Sources: TSCA**

(**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

3896 REFERENCES IN FILE CA (1907 TO DATE)

15 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA

3906 REFERENCES IN FILE CAPLUS (1907 TO DATE)

FILE 'CAPLUS' ENTERED AT 14:48:35 ON 19 SEP 2005

=> S RESTRICTION(W) (ENZYME OR ENDONUCLEASE);S L1;S L1,L2

98043 RESTRICTION

12880 RESTRICTIONS

110110 RESTRICTION

(RESTRICTION OR RESTRICTIONS)

749541 ENZYME

433271 ENZYMES

946960 ENZYME

(ENZYME OR ENZYMES)

26905 ENDONUCLEASE

8071 ENDONUCLEASES

31236 ENDONUCLEASE

(ENDONUCLEASE OR ENDONUCLEASES)

L2 31989 RESTRICTION(W) (ENZYME OR ENDONUCLEASE)

L3 3906 L1

3906 L1

L4 32300 (L1 OR L2)

=> S MUTAT?;S RECOGNITION SEQUENCE

L5 300124 MUTAT?

107976 RECOGNITION
 204 RECOGNITIONS
 108069 RECOGNITION
 (RECOGNITION OR RECOGNITIONS)
 657133 SEQUENCE
 467660 SEQUENCES
 777984 SEQUENCE
 (SEQUENCE OR SEQUENCES)
 L6 3737 RECOGNITION SEQUENCE
 (RECOGNITION(W) SEQUENCE)

=> S ALTER?;S METHYLASE OR METHYLTRANSFERASE
 L7 856688 ALTER?

2784 METHYLASE
 711 METHYLASES
 3003 METHYLASE
 (METHYLASE OR METHYLASES)
 15675 METHYLTRANSFERASE
 2611 METHYLTRANSFERASES
 16151 METHYLTRANSFERASE
 (METHYLTRANSFERASE OR METHYLTRANSFERASES)
 L8 18257 METHYLASE OR METHYLTRANSFERASE

=> S NON COGNATE
 744580 NON
 33 NONS
 744606 NON
 (NON OR NONS)
 10333 COGNATE
 143 COGNATES
 10441 COGNATE
 (COGNATE OR COGNATES)
 L9 219 NON COGNATE
 (NON(W) COGNATE)

=> S L9 AND L8
 L10 4 L9 AND L8

=> S L8 AND L10
 L11 4 L8 AND L10

=> D 1-4 CBIB ABS

L11 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN
 2005:187974 M.Ecori: enzyme studies. Gomez, Jose Antonio; Youngblood,
 Benjamin; Reich, Norbert O. (Department of Chemistry, UC Berkeley,
 Berkeley, CA, 94720, USA). Abstracts of Papers, 229th ACS National
 Meeting, San Diego, CA, United States, March 13-17, 2005, CHED-838.
 American Chemical Society: Washington, D. C. (English) 2005. CODEN:
 69GQMP.

AB Methyltransferases epigenetically modify DNA by adding a Me group to the major
 groove of cytosines and/or adenines in duplex DNA. Methyltransferases bend their
 DNA and flip out the target base in preparation for methylation. Thus, these
 enzymes are excellent models to test the importance of conformational transitions
 towards specificity. The Reich lab recently showed that M.EcoRI also
 intercalates its amino acids into the B-form DNA. This is a novel finding for
 this class of enzymes. We are now trying to determine how the different
 conformational mechanisms (bending, flipping, intercalation) contribute to this
 enzyme's discrimination between the noncognate and cognate sites. Fluorescence

Resonance Energy Transfer measures the distance between two dyes in the 20-60Å range. We prepared DNA with two dyes on the 5' ends of 14 bp duplexes, and determined the extent of protein-induced equilibrium changes, as well as the stopped-flow determination of bending kinetics. The hypothesis being tested is that the bending/flipping transitions are altered to the point that they become rate limiting. Affinity for the noncognate sites was determined using a modified gel shift assay in which the cognate sites compete against increasing concns. of the noncognate site. Preliminary results show intercalation and bending differ for the three noncognate sites, suggesting that different mechanisms of specificity are at play with different non-cognate sequences.

L11 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN

2004:1024316 Document No. 142:88708 Specificity in Protein-DNA Interactions: Energetic Recognition by the (Cytosine-C5)-methyltransferase from HhaI. Huang, Niu; MacKerell, Alexander D. (Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, MD, 21201, USA). Journal of Molecular Biology, Volume Date 2005, 345(2), 265-274 (English) 2004. CODEN: JMOBAK. ISSN: 0022-2836. Publisher: Elsevier B.V..

AB Sequence-specific interactions between proteins and DNA are essential for a variety of biol. functions. The (cytosine-C5)-methyltransferase from HhaI (M.HhaI) specifically modifies the second base in GCGC sequences, employing a base flipping mechanism to access the target base being chemical modified. The mechanism of sequence-specific recognition of M.HhaI is not evident on the basis of crystallog. structures, which suggests that recognition is linked to the flipping event itself, a process that may be referred to as energetic recognition. Using computational methods, it is shown that the free energy barriers to flipping are significantly higher in non-cognate vs. the cognate sequence, supporting the energetic recognition mechanism. Energetic recognition is imparted by two protein "selectivity filters" that function via a "web" of protein-DNA interactions in short-lived, high energy states present along the base flipping pathway. Other sequence-specific DNA binding proteins whose function involves significant distortion of DNA's conformation may use a similar recognition mechanism.

L11 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN

2003:571173 Document No. 139:113669 Alteration of restriction endonuclease specificity by genetic selection. Samuelson, James C.; Xu, Shuang-Yong (New England Biolabs, Inc., USA). PCT Int. Appl. WO 2003060152 A2 20030724, 51 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US542 20030109. PRIORITY: US 2002-PV347403 20020110.

AB Methods and compns. are provided for altering the DNA recognition and cleavage characteristics of an endonuclease without prior knowledge of the endonuclease's three-dimensional structure and/or amino acid residues responsible for activity and/or specificity. A protocol developed to achieve in vivo selection process includes one or more of the following steps: (1) generating a mutated endonuclease library within an expression vector or plasmid; (2) introducing the endonuclease library into prokaryotic host cells pre-modified with a non-cognate pattern of methylation; (3) pooling survivors and plasmid DNA from the cells; (4) isolating active endonuclease clones by culturing individual colonies for a short time at a low temperature and preparing plasmid DNA from these cultures; (5) introducing individual plasmid isolates (or pooled plasmid DNA) into a DNA damage indicator strain which is pre-modified with the same pattern of methylation as in step (2). The stringent selection method allows rapid screening of an estimated

107 variants in one round. The method is exemplified by increasing the substrate specificity of *Bacillus stearothermophilus* Y406 endonuclease BstYI (5'-RGATCY-3') to single site recognition (5'-AGATCT-3').

L11 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN

1999:632011 Document No. 132:19506 DNA nicks inflicted by restriction endonucleases are repaired by a RecA- and RecB-dependent pathway in *Escherichia coli*. Heitman, Joseph; Ivanenko, Tatyana; Kiss, Antal (Departments of Genetics, Pharmacology and Cancer Biology, Microbiology, Duke University Medical Center, Durham, NC, 27710, USA). *Molecular Microbiology*, 33(6), 1141-1151 (English) 1999. CODEN: MOMIEE. ISSN: 0950-382X. Publisher: Blackwell Science Ltd..

AB Two mutants of the EcoRI endonuclease (R200K and E144C) predominantly nick only one strand of the DNA substrate. Temperature sensitivity of the mutant enzymes allowed the authors to study the consequences of inflicting DNA nicks at EcoRI sites in vivo. Expression of the EcoRI endonuclease mutants in the absence of the EcoRI methyltransferase induces the SOS DNA repair response and greatly reduces viability of *recA56*, *recB21* and *lexA3* mutant strains of *Escherichia coli*. In parallel studies, overexpression of the EcoRV endonuclease in cells also expressing the EcoRV methyltransferase was used to introduce nicks at non-cognate EcoRV sites in the bacterial genome. EcoRV overprodn. was lethal in *recA56* and *recB21* mutant strains and moderately toxic in a *lexA3* mutant strain. The toxic effect of EcoRV overprodn. could be partially alleviated by introduction into the cells of multiple copies of the *E. coli* DNA ligase gene. These observations suggest that an increased number of DNA nicks can overwhelm the repair capacity of DNA ligase, resulting in the conversion of a proportion of DNA nicks into DNA lesions that require recombination for repair.

=> S (L5,L7) (6A)L8

L12 650 ((L5 OR L7)) (6A)L8

=> S L12 AND L4

L13 40 L12 AND L4

=> S (L5,L7) AND L6

L14 899 ((L5 OR L7)) AND L6

=>

=> S L14,L13

L15 932 (L14 OR L13)

=> S L13 AND L14

L16 7 L13 AND L14

=> S L16 NOT L11

L17 7 L16 NOT L11

=> D 1-7 CBIB ABS

L17 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2005 ACS on STN

2002:119325 Document No. 136:178936 Process for producing a polynucleotide encoding a restriction endonuclease. Janulaitis, Arvydas; Rimseliene, Renata; Lubys, Arvydas (Fermentas Ab, Lithuania). *Eur. Pat. Appl. EP 1179596 A1 20020213*, 44 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (English). CODEN: EPXXDW. APPLICATION: EP 2001-305859 20010706. PRIORITY: GB 2000-19744 20000810.

AB A process for producing a polynucleotide encoding a restriction endonuclease with an altered specificity. The process comprises of mutagenizing a polynucleotide encoding a restriction endonuclease with specificity for a recognition sequence so as to produce one or more mutated polynucleotides. The invention also relates

to the isolation of polynucleotide encoding a mutated restriction endonuclease with specificity for an altered recognition sequence by selecting a polynucleotide which expresses a restriction endonuclease with methylase specificity for the altered recognition sequence.

L17 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2005 ACS on STN

1998:542204 Document No. 129:227914 The cyanobacterium *Synechocystis* sp. strain PCC 6803 expresses a DNA methyltransferase specific for the recognition sequence of the restriction endonuclease PvuI. Scharnagl, Matthias; Richter, Stefan; Hagemann, Martin (FB Biologie, Universitat Rostock, Rostock, D-18051, Germany). *Journal of Bacteriology*, 180(16), 4116-4122 (English) 1998. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.

AB By use of restriction endonucleases, the DNA of the cyanobacterium *Synechocystis* sp. strain PCC 6803 was analyzed for DNA-specific methylation. Three different recognition sites of methyltransferases, a dam-like site including N6-methyladenosine and two other sites with methylcytosine, were identified, whereas no activities of restriction endonucleases could be detected in this strain. *Slr0214*, a *Synechocystis* gene encoding a putative methyltransferase that shows significant similarities to C5-methylcytosine-synthesizing enzymes, was amplified by PCR and cloned for further characterization. Mutations in *slr0214* were generated by the insertion of an *aphII* gene cassette. Analyses of chromosomal DNAs of such mutants demonstrated that the methylation pattern was changed. The recognition sequence of the methyltransferase was identified as 5'-CGATCG-3', corresponding to the recognition sequence of PvuI. The specific methyltransferase activity was significantly reduced in protein exts. obtained from mutant cells. Mutation of *slr0214* also led to changed growth characteristics of the cells compared to wild-type cells. These alterations led to the conclusion that the methyltransferase *Slr0214* might play a regulatory role in *Synechocystis*. The *Slr0214* protein was also overexpressed in *Escherichia coli*, and the purified protein demonstrated methyltransferase activity and specificity for PvuI recognition sequences in vitro. We propose the designation SynMI (*Synechocystis* methyltransferase I) for the *slr0214*-encoded enzyme.

L17 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2005 ACS on STN

1998:298608 Document No. 129:51408 Functional analysis of conserved motifs in type III restriction-modification enzymes. Saha, Swati; Ahmad, Ishtiaque; Reddy, Yeturu V. R.; Krishnamurthy, Vinita; Rao, Desirazu N. (Department Biochemistry, Indian Institute Science, Bangalore, 560012, India). *Biological Chemistry*, 379(4/5), 511-517 (English) 1998. CODEN: BICHF3. ISSN: 1431-6730. Publisher: Walter de Gruyter & Co..

AB EcoPII and EcoP15I are members of type III restriction-modification enzymes. DNA methyltransferases EcoPI and EcoP15I transfer a Me group from S-adenosyl-L-methionine (AdoMet) to the N6 position of the 2nd adenine residues in their recognition sequences, 5'-AGACC-3' and 5'-CAGCAG-3' resp. Here, the authors altered various residues in 2 highly conserved sequences, FxGxG (motif I) and DPPY (motif IV) in these proteins by site-directed mutagenesis. Using a mixture of in vivo and in vitro assays, the results on the mutational anal. of these methyltransferases demonstrated the universal role of motif I in AdoMet binding and a role for motif IV in catalysis. All 6 Cys residues in DNA methyltransferase EcoP15I were substituted with Ser and the role of the Cys residues in the EcoP15I methyltransferase-catalyzed reaction were assessed. The Res subunits of type III restriction enzymes share a distant sequence similarity with and contain the motifs characteristic of the DEAD box proteins. The authors also carried out site-directed mutagenesis of the conserved residues in 2 of the helicase motifs of restriction endonuclease EcoPII to investigate the role of motifs in DNA cleavage by this enzyme. The findings indicated that certain conserved residues in these motifs are involved in ATP hydrolysis whereas the other residues are involved in coupling restriction of DNA to ATP hydrolysis. These results form the

basis for a detailed structure-function anal. of EcoP1I and EcoP15I restriction enzymes.

L17 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2005 ACS on STN

1997:425658 Document No. 127:145770 Temperate *Myxococcus xanthus* phage Mx8 encodes a DNA adenine methylase, Mox. Magrini, Vincent; Salmi, Daniel; Thomas, David; Herbert, Stephen K.; Hartzell, Patricia L.; Youderian, Philip (Dep. Microbiol., Mol. Biol., Biochem., Univ. Idaho, Moscow, ID, 83844-3052, USA). *Journal of Bacteriology*, 179(13), 4254-4263 (English) 1997. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.

AB Temperate bacteriophage Mx8 of *Myxococcus xanthus* encapsidates terminally repetitious DNA, packaged as circular permutations of its 49-kbp genome. During both lytic and lysogenic development, Mx8 expresses a nonessential DNA methylase, Mox, which modifies adenine residues in occurrences of XhoI and PstI recognition sites, CTCGAG and CTGCAG, resp., on both phage DNA and the host chromosome. The *mox* gene is necessary for methylase activity in vivo, because an amber mutation in the *mox* gene abolishes activity. The *mox* gene is the only phage gene required for methylase activity in vivo, because ectopic expression of *mox* as part of the *M. xanthus* *mg1BA* operon results in partial methylation of the host chromosome. The predicted amino acid sequence of Mox is related most closely to that of the methylase involved in the cell cycle control of *Caulobacter crescentus*. The authors speculate that Mox acts to protect Mx8 phage DNA against restriction upon infection of a subset of natural *M. xanthus* hosts. One natural isolate of *M. xanthus*, the lysogenic source of related phage Mx81, produces a restriction endonuclease with the cleavage specificity of endonuclease BstBI.

L17 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2005 ACS on STN

1996:300709 Document No. 125:29460 Effect of methylation on the electrophoretic mobility of chromosomal DNA in pulsed-field agarose gels. Xydas, Steve; Lange, Christopher S.; Phil, D.; Neimark, Harold C. (Health Science Center Brooklyn, State University New York, Brooklyn, NY, 11203, USA). *Applied and Theoretical Electrophoresis*, 6(1), 43-47 (English) 1996. CODEN: ATELEM. ISSN: 0954-6642. Publisher: Electrophoresis Society.

AB Factors other than mol. weight are known to affect DNA electrophoretic mobility. DNA methylation has been found to affect the curvature of DNA, causing anomalous mobility in polyacrylamide gels; the effect of methylation on the mobility of large DNA mols. in agarose gels was unknown. Chromosomal DNA from *Mycoplasma capricolum*, a wall-less prokaryote which has a low intrinsic methylation rate, was methylated in agarose blocks by SssI methylase, a de novo methylase with a CpG recognition sequence. A surprising finding was that SssI methylase altered the structure of InCert, but not SeaKem Gold, agarose. Restriction enzyme anal. was used to estimate the extent of CpG methylation. DNA methylation was found to have no effect on the electrophoretic mobility of full-length chromosomal DNA (1120 kbp) in agarose gels. Therefore, methylation is not a source of error in PFGE-based size estimation for chromosomal DNA mols. <1.12 Mbp in agarose gels.

L17 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2005 ACS on STN

1993:421689 Document No. 119:21689 Construction of a *Neisseria gonorrhoeae* MS11 derivative deficient in NgoMI restriction and modification. Stein, Daniel C.; Chien, Roger; Seifert, H. Steven (Dep. Microbiol., Univ. Maryland, College Park, MD, 20742, USA). *Journal of Bacteriology*, 174(15), 4899-906 (English) 1992. CODEN: JOBAAY. ISSN: 0021-9193.

AB The gene encoding a methylase that modifies the sequence GCCGGC was cloned from *Neisseria gonorrhoeae* MS11. The corresponding restriction enzyme was also encoded by this clone. Sequence anal. demonstrated that the methylase shares sequence similarities with other cytosine methylases, but the sequence organization of *M. NgoMI* is different from that seen for other cytosine methylases. A deletion was introduced into the chromosome of *N. gonorrhoeae* MS11

to produce strain MUG701, a strain that is inactivated in both the methylase and the restriction genes. Although this strain no longer methylated its DNA at the NgoNI recognition sequence, cells were viable and had no other significant phenotypic changes. Transformation data indicated that MS11 does not produce enough restriction activity to block plasmid transformation in the gonococcus, even though restriction activity could be demonstrated in E. coli containing the cloned gene.

L17 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2005 ACS on STN

1984:587404 Document No. 101:187404 Alteration of apparent restriction endonuclease recognition specificities by

DNA methylases. Nelson, Michael; Christ, Chantal; Schildkraut, Ira (New England Biolabs, Inc., Beverly, MA, 01915, USA). Nucleic Acids Research, 12(13), 5165-73 (English) 1984. CODEN: NARHAD. ISSN: 0305-1048.

AB An in vitro method of altering the apparent cleavage specificities of restriction endonucleases was developed by using DNA modification methylases. This method was used to reduce the number of cleavage sites for 34 restriction endonucleases. In particular, single-site cleavages were achieved for NheI in Adeno-2 DNA and for AccI and HincII in pBR322 DNA by specifically methylating all but one recognition sequence.

=> S L4 AND L12

L18 40 L4 AND L12

=> S L18 NOT (L17,L10)

L19 32 L18 NOT ((L17 OR L10))

=> D L19 1-32 TI

=> D 1,21,22,27 CBIB ABS

L19 ANSWER 1 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN

2004:936088 Document No. 141:389850 Methods for altering the cleavage specificity of a type IIG restriction endonuclease.

Xu, Shuang-Yong; Kobbe, Daniela; Zhu, Zhenyu; Samuelson, James (USA).

U.S. Pat. Appl. Publ. US 2004219584 A1 20041104, 34 pp., Cont.-in-part of

U.S. Ser. No. 150,028, abandoned. (English). CODEN: USXXCO.

APPLICATION: US 2004-800946 20040315. PRIORITY: US 2000-2000/693146

20001020; US 2002-2002/150028 20020517.

AB The current invention provides methods for altering the cleavage specificity of a type IIG restriction endonuclease. Type IIG restriction endonuclease contains a cleavage domain adjacent to a methylase domain, the methylase domain located adjacent to a specificity domain. The method includes ligating a first DNA sequence and a second DNA sequence to form a fusion DNA, wherein (i) the first DNA sequence comprises a DNA segment encoding a catalytic domain and an N-terminal portion of a methylase domain of a first Type IIG restriction endonuclease, and (ii) the second DNA sequence, comprises a DNA segment encoding a specificity domain and a C-terminal portion of a methylase domain of a second Type IIG restriction endonuclease; such that the ligation occurs between sequences encoding the methylase domain. The host cell is transformed with fusion DNA to express a Type IIG restriction endonuclease with altered cleavage specificity.

L19 ANSWER 21 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN

1992:646170 Document No. 117:246170 Methyltransferases as tools to alter the specificity of restriction

endonucleases. Sobral, Bruno W. S.; McClelland, Michael

(California Inst. Biol. Res., La Jolla, CA, USA). Methods in Molecular

Biology (Totowa, NJ, United States), 12(Pulsed-Field Gel Electrophor.),

159-72 (English) 1992. CODEN: MMBIED. ISSN: 1064-3745.

AB Pulsed-field gel electrophoresis (PFGE) has allowed the resolution of very large DNA fragments from any organism. To apply PFGE to practical problems, such as

genetic mapping and map-based gene cloning, it is necessary to specifically generate large DNA fragments that can then be separated by PFGE. Ideally, restriction enzymes would exist that could generate DNA fragments of desired sizes. Other factors being equal, and supposing that DNA sequences were random, then enzymes with larger target sequences should produce larger DNA fragments. In practice, no restriction enzymes are known to have larger than 8-bp-long target sites and, of course, DNA sequences are not random. These realities severely limit the observed sizes of DNA fragments produced by restriction enzymes acting on genomic DNA, particularly in the case of eukaryotic genomes, which are large and complex. Here, the authors describe enzymic strategies to generate large DNA fragments and statistical tools that can aid researchers in choosing the restriction enzymes that are most likely to generate large fragments in the genome in question, if a sequence data base can be investigated.

L19 ANSWER 22 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN

1992:445303 Document No. 117:45303 Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Li, En; Bestor, Timothy H.; Jaenisch, Rudolf (Whitehead Inst. Biomed. Res., Cambridge, MA, 02142, USA). Cell (Cambridge, MA, United States), 69(6), 915-26 (English) 1992. CODEN: CELLB5. ISSN: 0092-8674.

AB Gene targeting in embryonic stem (ES) cells has been used to mutate the murine DNA methyltransferase gene. ES cell lines homozygous for the mutation were generated by consecutive targeting of both wild-type alleles; the mutant cells were viable and showed no obvious abnormalities with respect to growth rate or morphol., and had only trace levels of DNA methyltransferase activity. A quant. end-labeling assay showed that the level of m5C in the DNA of homozygous mutant cells was about one-third that of wild-type cells, and Southern blot anal. after cleavage of the DNA with a methylatino-sensitive restriction endonuclease revealed substantial demethylation of endogenous retroviral DNA. The mutation was introduced into the germline of mice and found to cause a recessive lethal phenotyhpe. Homozygous embryos were stunted, delayed in development, and did not survive past mid-gestation. The DNA of homozygous embryos showed a reduction of the level of m5C similar to that of homozygous ES cells. These results indicate that while a 3-fold reduction in levels of genomic m5C has no detectable effect on the viability or proliferation of ES cells in culture, a similar reduction of DNA methylation in embryos causes abnormal development and embryonic lethality.

L19 ANSWER 27 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN

1988:163633 Document No: 108:163633 The use of DNA methylases to alter the apparent recognition specificities of restriction endonucleases. Nelson, Michael; Schildkraut, Ira (New England Biolabs, Inc., Beverly, MA, 01915, USA). Methods in Enzymology, 155(Recomb. DNA, Pt. F), 41-8 (English) 1987. CODEN: MENZAU. ISSN: 0076-6879.

AB A review, with 9 refs., on the DNA methylase/endonuclease combinations which have generated new cleavage specificities. Reagents and procedures are also reviewed.

	L #	Hits	Search Text	DBs
1	L1	55962	RESTRICTION ADJ (ENZYME OR ENDONUCLEASE)	US- PGPUB; USPAT
2	L2	3420	MUTAT?	US- PGPUB; USPAT
3	L3	63003	ALTER?	US- PGPUB; USPAT
4	L4	19229	RECOGNITION ADJ SITE	US- PGPUB; USPAT
5	L5	212	NON COGNATE	US- PGPUB; USPAT
6	L6	5295	METHYLASE OR METHYLTRANSFERASE	US- PGPUB; USPAT
7	L7	4	(L2 OR L3) NEAR L4	US- PGPUB; USPAT
8	L8	27	L5 NEAR L6	US- PGPUB; USPAT
9	L9	27	L8 AND L1	US- PGPUB; USPAT